

## CLAIMS:

1. An isolated MUC1-specific binding member comprising an antigen binding domain, wherein the antigen binding domain comprises a region comprising the amino acid sequence of the formula:

$X_1 X_2 \text{ His Thr Gly } X_3 \text{ Gly Val Trp } X_4 \text{ Pro } X_5 X_6 X_7$  (SEQ ID NO:28),

5 wherein  $X_1$  is Ala, Ser, Thr, or Val;

$X_2$  is Lys, Ile Arg, or Gln;

$X_3$  is Gly, Arg, Val, Glu, Ser, or Ala;

$X_4$  is Asp or Asn;

$X_5$  is Ile, Leu, Met, Phe, or Val;

10  $X_6$  is Asp, Gly, Lys, Asn, Ala, His, Arg, Ser, Val, or Tyr; and

$X_7$  is Tyr, His, Lys, Asn, Asp, Ser, Pro.

2. The MUC1-specific binding member according to Claim 1, wherein the variable region comprises the amino acid sequence selected from the group consisting of:

15  $\begin{matrix} A & L & H & T & G & G & V & T & D & P & I & D & Y \\ \times & \text{Ala} & \text{Lys} & \text{His} & \text{Thr} & \text{Gly} & \text{Gly} & \text{Gly} & \text{Val} & \text{Trp} & \text{Asp} & \text{Pro} & \text{Ile} & \text{Asp} & \text{Tyr} \end{matrix}$  (amino acids 97-110 of SEQ ID NO:3);

Ala Lys His Thr Gly Arg Gly Val Trp Asp Pro Ile Gly Tyr (SEQ ID NO:29);

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Lys His (SEQ ID NO:30);

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Gly Tyr (SEQ ID NO:31); and

20 Ala Ile His Thr Gly Gly Gly Val Trp Asp Pro Ile Lys Tyr (SEQ ID NO:32).

3. An isolated MUC1-specific binding member comprising an antigen binding domain comprising an antibody  $V_L$  region comprising the amino acid sequence of SEQ ID NO:1 [or portion thereof], and an antibody  $V_H$  region comprising the amino acid sequence of SEQ ID NO:3 [or portion thereof].

4. A MUC1-specific binding member comprising an antigen binding domain, wherein the antigen binding domain comprises a CDR of an antibody  $V_L$  or  $V_H$  region, wherein said CDR has an amino acid sequence selected from the group consisting of amino acids 24 to 39 of SEQ ID NO:1, amino acids 55 to 61 of SEQ ID NO:1, amino acids 94 to 102 of SEQ ID NO:1, amino acids 31 to 35 of SEQ ID NO:3, amino acids 50 to 66 of SEQ ID NO:3, amino acids 99 to 110 of SEQ ID NO:3 [conservatively substituted sequences of any of the preceding sequences, and combinations thereof].

- 107 5. The MUC1-specific binding member according to any of Claims 1, 2, 3, or 4, wherein said MUC1-specific binding member is a fusion protein.
- 107 6. The MUC1-specific binding member according to any one of Claims 1, 2, 3, or 4, further  
5 comprising a detectable label or tag.
- 107 7. The MUC1-specific binding member according to Claim 6, wherein the detectable label or tag is selected from the group consisting of epitope tags, fluorescent labels, radioactive labels, heavy metals, anti-cancer drugs, toxins, and magnetic resonance imaging labels.
- 10 8. The MUC1-specific binding member according to any one of Claims 1, 2, 3, or 4, wherein the MUC1-specific binding member is an antibody molecule selected from the group consisting of immunoglobulins, Fab antibodies, F(ab')<sub>2</sub> antibodies, diabodies, scFv antibodies, double scFv, Fv molecules, dAb, immunocytokine molecules, and immunotoxin molecules.
- 15 9. The MUC1-specific immunocytokine according to Claim 8, comprising the amino acid sequence of SEQ ID NO (5).
- 10 10. The MUC1-specific immunocytokine according to Claim 9, further comprising a detectable  
20 label or tag.
11. The MUC1-specific binding member according to Claim 10, wherein the detectable label or tag is selected from the group consisting of, epitope tags, fluorescent labels, radioactive labels, and magnetic resonance imaging labels.
- 25 12. The MUC1-specific immunoglobulin according to Claim 8, comprising a light chain polypeptide comprising the amino acid sequence of SEQ ID NO (24) and a heavy chain polypeptide comprising the amino acid sequence of SEQ ID NO (26).
- 30 13. The MUC1-specific immunoglobulin according to Claim 12, further comprising a detectable label or tag.

14. The MUC1-specific binding member according to Claim 13, wherein the detectable label or tag is selected from the group consisting of enzymes, epitope tags, fluorescent labels, radioactive labels, heavy metals, anti-cancer drugs, toxins, and magnetic resonance imaging labels.

5 15. A MUC1-specific binding member comprising an antibody antigen binding domain comprising a heavy chain variable region, or CDR thereof, from the DP47 germ line. 112, 152

16. A MUC1-specific binding member comprising an antibody antigen binding domain comprising a light chain variable region, or a CDR thereof, from the DPK15 germ line.

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17. A MUC1-specific binding member comprising an antibody antigen binding domain comprising a heavy chain variable region, or CDR thereof, from the DP47 germ line and a light chain variable region, or CDR thereof, from the DPK15 germ line.

15 18. A MUC1-specific binding member comprising an amino acid sequence that is about 70% or more homologous to any of the amino acid sequences of Claims 1, 2, 3, or 4.

19. A MUC1-specific binding member comprising an amino acid sequence that is about 80% or more homologous to any of the amino acid sequences of Claims 1, 2, 3, or 4.

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20. A MUC1-specific binding member comprising an amino acid sequence that is about 90% or more homologous to any of the amino acid sequences of Claims 1, 2, 3, or 4.

21. A MUC1-specific binding member comprising an amino acid sequence that is about 95% or more homologous to any of the amino acid sequences of Claims 1, 2, 3, or 4.

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22. A MUC1-specific binding member comprising an amino acid sequence that is about 97% or more homologous to any of the amino acid sequences of Claims 1, 2, 3, or 4.

23. A MUC1-specific binding member comprising an amino acid sequence that is about 99% or more homologous to any of the amino acid sequences of Claims 1, 2, 3, or 4.

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24. A polypeptide molecule comprising an amino acid sequence that is about 70% or more homologous to an amino acid sequence selected from the group consisting of SEQ ID NO:1, amino acids 24 to 39 of SEQ ID NO:1, amino acids 55 to 61 of SEQ ID NO:1, amino acids 94 to 102 of SEQ ID NO:1, SEQ ID NO:3, amino acids 31 to 35 of SEQ ID NO:3, amino acids 50 to 66 of SEQ ID NO:3, amino acids 99 to 110 of SEQ ID NO:3, and SEQ ID NO:5.

25. A polypeptide molecule comprising an amino acid sequence that is about 80% or more homologous to an amino acid sequence selected from the group consisting of SEQ ID NO:1, amino acids 24 to 39 of SEQ ID NO:1, amino acids 55 to 61 of SEQ ID NO:1, amino acids 94 to 102 of SEQ ID NO:1, SEQ ID NO:3, amino acids 31 to 35 of SEQ ID NO:3, amino acids 50 to 66 of SEQ ID NO:3, amino acids 99 to 110 of SEQ ID NO:3, and SEQ ID NO:5.

26. A polypeptide molecule comprising an amino acid sequence that is about 90% or more homologous to an amino acid sequence selected from the group consisting of SEQ ID NO:1, amino acids 24 to 39 of SEQ ID NO:1, amino acids 55 to 61 of SEQ ID NO:1, amino acids 94 to 102 of SEQ ID NO:1, SEQ ID NO:3, amino acids 31 to 35 of SEQ ID NO:3, amino acids 50 to 66 of SEQ ID NO:3, amino acids 99 to 110 of SEQ ID NO:3, and SEQ ID NO:5.

27. A polypeptide molecule comprising an amino acid sequence that is about 95% or more homologous to an amino acid sequence selected from the group consisting of SEQ ID NO:1, amino acids 24 to 39 of SEQ ID NO:1, amino acids 55 to 61 of SEQ ID NO:1, amino acids 94 to 102 of SEQ ID NO:1, SEQ ID NO:3, amino acids 31 to 35 of SEQ ID NO:3, amino acids 50 to 66 of SEQ ID NO:3, amino acids 99 to 110 of SEQ ID NO:3, and SEQ ID NO:5.

28. A polypeptide molecule comprising an amino acid sequence that is about 97% or more homologous to an amino acid sequence selected from the group consisting of SEQ ID NO:1, amino acids 24 to 39 of SEQ ID NO:1, amino acids 55 to 61 of SEQ ID NO:1, amino acids 94 to 102 of SEQ ID NO:1, SEQ ID NO:3, amino acids 31 to 35 of SEQ ID NO:3, amino acids 50 to 66 of SEQ ID NO:3, amino acids 99 to 110 of SEQ ID NO:3, and SEQ ID NO:5.

29. A polypeptide molecule comprising an amino acid sequence that is about 99% or more homologous to an amino acid sequence selected from the group consisting of SEQ ID NO:1, amino acids 24 to 39 of SEQ ID NO:1, amino acids 55 to 61 of SEQ ID NO:1, amino acids 94 to 102 of SEQ

ID NO:1, SEQ ID NO:3, amino acids 31 to 35 of SEQ ID NO:3, amino acids 50 to 66 of SEQ ID NO:3, amino acids 99 to 110 of SEQ ID NO:3, and SEQ ID NO:5.

30. An isolated polynucleotide molecule comprising a nucleotide sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO:1, amino acids 24 to 39 of SEQ ID NO:1, amino acids 55 to 61 of SEQ ID NO:1, amino acids 94 to 102 of SEQ ID NO:1, SEQ ID NO:3, amino acids 31 to 35 of SEQ ID NO:3, amino acids 50 to 66 of SEQ ID NO:3, amino acids 99 to 110 of SEQ ID NO:3, SEQ ID NO:5, and combinations thereof.

31. An isolated polynucleotide molecule comprising a nucleotide sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO:24 and SEQ ID NO:26.

32. An isolated polynucleotide molecule encoding a V<sub>L</sub> region comprising a nucleotide sequence of SEQ ID NO:2 or degenerate sequences thereof.

33. An isolated polynucleotide molecule encoding a V<sub>L</sub> region comprising a nucleotide sequence which is about 70% or more homologous to the sequence of SEQ ID NO:2.

34. An isolated polynucleotide molecule encoding a V<sub>L</sub> region comprising a nucleotide sequence which is about 80% or more homologous to the sequence of SEQ ID NO:2.

35. An isolated polynucleotide molecule encoding a V<sub>L</sub> region comprising a nucleotide sequence which is about 90% or more homologous to the sequence of SEQ ID NO:2.

36. An isolated polynucleotide molecule encoding a V<sub>L</sub> region comprising a nucleotide which is about 95% or more homologous to the sequence of SEQ ID NO:2.

37. An isolated polynucleotide molecule encoding a V<sub>L</sub> region comprising a nucleotide sequence which is about 97% or more homologous to the sequence of SEQ ID NO:2.

38. An isolated polynucleotide molecule encoding a V<sub>L</sub> region comprising a nucleotide sequence which is about 99% or more homologous to the sequence of SEQ ID NO:2.

39. An isolated polynucleotide molecule encoding a V<sub>H</sub> region comprising a nucleotide sequence of SEQ ID NO:4, or degenerate sequences thereof.

40. An isolated polynucleotide molecule encoding a V<sub>H</sub> region comprising a nucleotide which is about 70% homologous to the sequence of SEQ ID NO:4.

41. An isolated polynucleotide molecule encoding a V<sub>H</sub> region comprising a nucleotide which is about 80% homologous to the sequence of SEQ ID NO:4.

42. An isolated polynucleotide molecule encoding a V<sub>H</sub> region comprising a nucleotide which is about 90% homologous to the sequence of SEQ ID NO:4.

43. An isolated polynucleotide molecule encoding a V<sub>H</sub> region comprising a nucleotide which is about 95% homologous to the sequence of SEQ ID NO:4.

44. An isolated polynucleotide molecule encoding a V<sub>H</sub> region comprising a nucleotide which is about 97% homologous to the sequence of SEQ ID NO:4.

45. An isolated polynucleotide molecule encoding a V<sub>H</sub> region comprising a nucleotide which is about 99% homologous to the sequence of SEQ ID NO:4.

46. An isolated polynucleotide molecule encoding a CDR of an antibody variable region comprising a nucleotide sequence selected from the group consisting of nucleotides 70 to 117 of SEQ ID NO:2, nucleotides 163 to 183 of SEQ ID NO:2, nucleotides 280 to 306 of SEQ ID NO:2, nucleotides 91 to 105 of SEQ ID NO:4, nucleotides 148 to 198 of SEQ ID NO:4, nucleotides 295 to 330 of SEQ ID NO:4, degenerate sequences of any of the preceding CDR coding sequences, and combinations thereof.

47. An isolated polynucleotide molecule comprising a nucleotide sequence that is about 60% or more homologous to a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, nucleotides 70 to 117 of SEQ ID NO:2, nucleotides 163 to 183 of SEQ ID NO:2, nucleotides 280 to 306 of SEQ ID NO:2, nucleotides 91 to 105 of SEQ ID NO:4, nucleotides 148 to 198 of SEQ ID NO:4, and nucleotides 295 to 330 of SEQ ID NO:4.

48. An isolated polynucleotide molecule comprising a nucleotide sequence that is about 70% or more homologous to a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, nucleotides 70 to 117 of SEQ ID NO:2, nucleotides 163 to 183 of SEQ ID NO:2, nucleotides 280 to 306 of SEQ ID NO:2, nucleotides 91 to 105 of SEQ ID NO:4, nucleotides 148 to 198 of SEQ ID NO:4, and nucleotides 295 to 330 of SEQ ID NO:4.

49. An isolated polynucleotide molecule comprising a nucleotide sequence that is about 80% or more homologous to a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, nucleotides 70 to 117 of SEQ ID NO:2, nucleotides 163 to 183 of SEQ ID NO:2, nucleotides 280 to 306 of SEQ ID NO:2, nucleotides 91 to 105 of SEQ ID NO:4, nucleotides 148 to 198 of SEQ ID NO:4, and nucleotides 295 to 330 of SEQ ID NO:4.

50. An isolated polynucleotide molecule comprising a nucleotide sequence that is about 90% or more homologous to a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, nucleotides 70 to 117 of SEQ ID NO:2, nucleotides 163 to 183 of SEQ ID NO:2, nucleotides 280 to 306 of SEQ ID NO:2, nucleotides 91 to 105 of SEQ ID NO:4, nucleotides 148 to 198 of SEQ ID NO:4, and nucleotides 295 to 330 of SEQ ID NO:4.

51. An isolated polynucleotide molecule comprising a nucleotide sequence that is about 95% or more homologous to a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, nucleotides 70 to 117 of SEQ ID NO:2, nucleotides 163 to 183 of SEQ ID NO:2, nucleotides 280 to 306 of SEQ ID NO:2, nucleotides 91 to 105 of SEQ ID NO:4, nucleotides 148 to 198 of SEQ ID NO:4, and nucleotides 295 to 330 of SEQ ID NO:4.

52. An isolated polynucleotide molecule comprising a nucleotide sequence that is about 97% or more homologous to a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, nucleotides 70 to 117 of SEQ ID NO:2, nucleotides 163 to 183 of SEQ ID NO:2, nucleotides 280 to 306 of SEQ ID NO:2, nucleotides 91 to 105 of SEQ ID NO:4, nucleotides 148 to 198 of SEQ ID NO:4, and nucleotides 295 to 330 of SEQ ID NO:4.

53. An isolated polynucleotide molecule encoding a MUC1-specific binding member comprising the nucleotide sequence of SEQ ID NO:6.

54. The isolated polynucleotide molecule according to any one of Claims 30-53, wherein the polynucleotide molecule is a molecule selected from the group consisting of linear polynucleotide molecules, plasmids, phagemids, bacteriophage vectors, yeast display vectors, and eukaryotic viral  
5 vectors.

55. A method of diagnosing cancer in an individual comprising:  
providing a biological sample from the individual;  
contacting the biological sample from the individual with a MUC1-specific binding member  
10 according to any one of Claims 1-23, conservatively substituted versions of any of the preceding sequences, and combinations thereof; and  
detecting binding of said MUC1-specific binding member to MUC1 in the biological sample of the individual.

15 56. The method of diagnosing cancer in an individual according to Claim 55, wherein the cancer is adenocarcinoma.

57. The method of diagnosing cancer in an individual according to Claim 55, wherein the biological sample from the individual is selected from the group consisting of cells, blood, lymph,  
20 urine, mammary tissue, ovary tissue, lung tissue, bladder tissue, and combinations thereof.

58. The method of diagnosing cancer in an individual according to Claim 55, wherein the binding of said MUC1-specific binding member to MUC1 is detected by a detection means selected from the group consisting of enzyme-linked immunosorbent assay, magnetic resonance imaging, scintillation  
25 counting, and X-ray film.

59. A method of treating cancer in an individual comprising:  
administering to the individual in need of treatment thereof a MUC1-specific binding member according to any one of Claims 1-23, conservatively substituted versions of any of the preceding  
30 sequences, and combinations thereof.

60. The method of treating cancer in an individual according to Claim 59, wherein the cancer is adenocarcinoma.



61. The method of treating cancer in an individual according to Claim 59, further comprising administering a cytokine to the individual before, contemporaneously with, or after administering the MUC1-specific binding member.

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62. The method of treating cancer in an individual according to Claim 59, wherein the cancer is present in tissue of the breast, ovary, lung, or bladder of the individual.

63. An *ex vivo* method of treating cancer in an individual comprising:

10 obtaining a body fluid containing MUC1 and/or MUC1-expressing cancer cells from an individual;

contacting the body fluid with an immobilized MUC1-specific binding member according to any one of Claims 1-23, conservatively substituted versions of any of the preceding sequences, and combinations thereof;

15 collecting the body fluid not bound to the immobilized MUC1-specific binding member; and

returning the collected body fluid not bound to the immobilized MUC1-specific binding member to the individual.

20 64. The *ex vivo* method of treating cancer according to Claim 63, further comprising the step of adding one or more therapeutic agents to the body fluid prior to returning the fluid to the individual.

65. The *ex vivo* method of treating cancer according to Claim 63, wherein the body fluid is selected from the group consisting of bone marrow, blood, and peripheral blood stem cells.

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66. The *ex vivo* method of treating cancer according to Claim 63, wherein the cancer is adenocarcinoma.

30 67. The *ex vivo* method of treating cancer according to Claim 63, wherein the anti-cancer reagent is a MUC1-specific binding member.

68. A method of making a MUC1-specific binding member comprising:  
 preparing an expression vector comprising a polynucleotide sequence according to any of  
Claims 30-54, conservatively substituted versions of any of the preceding sequences, and combinations  
 thereof;

5 inserting said expression vector into a host cell; and  
 culturing said host cell under conditions in which the MUC1-specific binding member is  
 expressed from the expression vector.

69. The method of making a MUC1-specific binding member according to Claim 68, wherein the  
 10 MUC1-specific binding member is selected from the group consisting of an immunoglobulin, a Fab  
 antibody, F(ab')<sub>2</sub> antibody, a diabody, a scFv, a double scFv, a dAb, a Fv, an immunotoxin, and an  
 immunocytokine.